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In vitro antimicrobial activity of plant extracts of semi-arid region of Paraíba, PB, Brazil

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Abstract

Objective: To evaluate the antimicrobial activity of ethanol extracts of *Schinus terebintifolius* Raddi (aroeira-da-praia), *Syderoxylum obtusifolium* Roem et Schult. (Quixabeira), *Bauhinia forficata* Linn (mororó), *Cnidoscolus urens* (L) Arthur (urtiga-branca), *Pseudobombax marginatum* (embiratanha), *Anadenanthera macrocarpa* (Benth) Brenan (angico), *Maytenus rigida* Mart (bom nome) and *Spondias tuberosa* A. Cam. (*S. tuberosa*) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mutans*, *Candida albicans*, *Candida tropicalis* and *Candida krusei.*

Methods: We used the agar disk diffusion technique in triplicate. After 48 h incubation at 37 °C the analysis and measurements of inhibition halos were performed.

Results: We found antimicrobial activity of extracts from aroeira-da-praia, mororó, angico, bom-nome and umbuzeiro against *E. faecalis*. *S. aureus* showed no sensitivity only to embiratanha and urtiga-branca. No substance showed antimicrobial activity against *P. aeruginosa, S. mutans, E. coli* and *Candida tropicalis*.

Conclusion: Of the extracts tested, only the urtiga-branca showed no antimicrobial activity while mororó showed activity for most microorganisms analyzed.

Keywords: Plant extracts; antimicrobial action; microorganisms

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Estudo *in vitro* da atividade antimicrobiana de extratos de plantas do semiárido da Paraíba, PB, Brasil

Resumo

Objetivo: Avaliar a ação antimicrobiana dos extratos etanólicos da *Schinus terebintifolius Raddi* (aroeira-da-praia), Syderoxylum obtusifolium Roem et Schult. (quixabeira), *Bauhínia forficata* Linn (mororó), *Cnidoscolus urens* (L) *Arthur* (urtiga-branca), *Pseudobombax marginatum* (embiratanha), *Anadenanthera macrocarpa* (Benth) Brenan (angico), *Maytenus rigida* Mart (bom-nome) e do *Spondias tuberosa* (umbuzeiro) contra *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococus mutans*, *Candida albicans*, *Candida tropicalis* e *Candida krusei*.

Métodos: Foi utilizada a técnica disco difusão em ágar, em triplicata. Após 48 horas de incubação a 37ºC, foram realizadas as análises e medições dos halos de inibição.

Resultados: Os resultados demonstraram atividade antimicrobiana dos extratos de aroeira-da-praia, mororó, angico, bom-nome e umbuzeiro contra o *E. faecalis*. O *S. aureus* não apresentou sensibilidade apenas para a embiratanha e urtiga-branca. Nenhuma substância mostrou atividade antimicrobiana contra o *P. aeruginosa*, *S. mutans* e a *Candida tropicalis*.

Conclusão: Dos extratos analisados, apenas a urtiga-branca não apresentou atividade antimicrobiana e o mororó foi o que apresentou atividade para a maioria dos microrganismos analisados.

Palavras-chave: Extratos vegetais; ação antimicrobiana; microrganismos

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Introduction

In recent years there has been a growing interest in the scientific community by medicinal plants and herbs, since they have therapeutic and economic potential, especially targeted by the pharmaceutical industry, which makes the prospect of new products with fewer side effects than existing drugs [1-3].

In dentistry, there is a current trend for investment in research aimed at the development of biocompatible substances, especially among those that will come into direct contact with the tissues [4]. In this context, the herbal medicine has evolved markedly in recent years and has stimulated the evaluation of different plant products with therapeutic properties in Dentistry [5,6].

The bark extract from *Schinus terebinthifolius* Raddi has been used as anti-inflammatory and antimicrobial, however a better assessment of the risks and benefits of the internal use of these plants is necessary [7]. Studies have been published revealing the therapeutic properties of Quixabeira (*Syderoxylum obtusifolium Roem* et Schult.), which had been used in folk medicine in Northeast Brazil, in the treatment of inflammations of the female genitourinary system [8] and oral infections [9]. The mororó (*Bauhinia forficata* linn) has several drug-therapeutic properties, including hypoglycemic activity [10]. From Angico (*Anadenanthera colubrina* Brenan) stands out the tannin, which has been used as healing, anti-inflammatory and astringent solution. The bark and root of bom-nome (*Maytenus rigida* Mart.) has been indicated for the treatment of infections and inflammatory processes [11]. The urtiga-branca (*Cnidoscolus urens* (L) Arthur) is used as anti-inflammatory in the urinary and ovarian tracts, and the umbuzeiro (*Spondias tuberosa* Arruda) as ophthalmic drug [8].

This study aimed at evaluating the antimicrobial activity of different semi-arid plants against bacteria and fungi commonly found in the oral cavity.

Methods

A study was conducted to evaluate the *in vitro* antimicrobial activity of hydroalcoholic extract from the bark of eight plants collected from the semiarid region of Paraíba, PB, Brazil, namely: *Schinus terebintifolius Raddi* (aroeira-da-praia), S*yderoxylum obtusifolium Roem* et Schult. (quixabeira), *Bauhínia forficata* Linn (mororó), *Cnidoscolus urens* (L) Arthur (urtiga-branca), *Pseudobombax marginatum* (embiratanha), *Anadenanthera macrocarpa* (Benth) Brenan (angico), *Maytenus rigida* Mart (bom-nome), *Spondias tuberosa* A. Câm. (umbuzeiro), against standard strains American Type Culture Collection (ATCC) of *Escherichia* coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 18804), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 34135), *Candida tropicalis* (ATCC 13803), *Enterococcus faecalis (*ATCC 29212) e *Streptococcus mutans* (ATCC 25175), provided by Fundação Oswaldo Cruz (FIOCRUZ/RJ).

The hydroalcoholic extract from the bark of selected plants were obtained by steeping method, with immersion of 200 g dry plant material in 1000 mL of 70% ethyl alcohol for five days. To obtain the dry plant material, barks were placed in Kraft-type paper bags and taken into the oven with air renewal and circulation at 40°C until moisture stabilization. Elapsed time of maceration, the extract was filtered and packaged in amber glass flasks, wrapped in aluminum foil, labeled, identified and stored in low light environment.

Strains were provided lyophilized by FIOCRUZ and kept under refrigeration until use. Bacteria reactivation occurred in inclined tubes containing nutrient agar, immediately after opening the protective envelope. This tube, called the "motherboard" (2nd passage) were incubated in culture at 37 °C for 24 hours and then kept in the refrigerator for up to 30 days.

From the "motherboard" was performed a new passage of bacteria (3rd passage), whose 24-hour lyophilisate was used for preparing microbial suspensions. For this, a verge of the culture grown in the inclined medium has been transferred from each microorganism species, using handle sterile test tube containing 3 mL of sterile saline solution. Each suspension was homogenized in vortex for 1 minute until reaching 0.5 McFarland scale (1.5×10^8 CFU/mL). The microbial inoculum was standardized before use by means of a spectrophotometer (Coleman model 35D), with 25% transmittance at 580 nm wavelength in order to obtain a microbial preparation with final concentration between 10^6 and 10^8 CFU/mL.

Inocula were seeded on Mueller Hinton agar plates (*E. faecalis, E. coli, P. aeruginosa* and *S. aureus*), blood agar (*S. mutans*) and Sabouraud agar (*Candida* spp) using sterile swab by depletion technique.

Ten disks were accommodated on each plate, where each one received 10 mL extract with the help of a micropipette. Plates were incubated in culture at 37 °C for 48 hours, time which the reading of inhibition halos was performed, making use of two perpendicular measurements with digital calipers. The experiment was performed in triplicate.

Results

Of the extracts tested, only the urtiga-branca showed no antimicrobial activity, and mororó showed the activity against most microorganisms. *E. faecalis* showed sensitivity to the aroeira-da-praia, mororó, angico, bom-nome and umbuzeiro, and extract from bom-nome showed the highest average of inhibition halo with 8.3 mm. The *S. aureus* was sensitive to most extracts. *P. aeruginosa, S. mutans, E. coli and C. tropicalis* were resistant to all extracts analyzed. Embiratanha and mororó showed antimicrobial activity against at least one *Candida* species except *C. tropicalis* (Tables 1 and 2).

Table 1. Average inhibition halos (mm) of crude extracts from plants by the technique of agar disk diffusion against various bacteria after 48 hours incubation at 37 °C.

Plant	*E. faecalis*	*S. aureus*	*S. mutans*	*E. coli*	*P. aeruginosa*
Angico	6.6±1.1	12.0±0.0	0.0	0.0	0.0
Aroeira	8.0±0.0	12.0±0.0	0.0	0.0	0.0
Bom-Nome	8.3±0.6	12.0±0.0	0.0	0.0	0.0
Embiratanha	0.0	0.0	0.0	0.0	0.0
Mororó	8.0±0.0	12.6±0.6	0.0	0.0	0.0
Quixabeira	0.0	8.0±0.0	0.0	0.0	0.0
Umbuzeiro	8.0±0.0	12.0±0.0	0.0	0.0	0.0
Urtiga-Branca	0.0	0.0	0.0	0.0	0.0
0.12% Chlorhexidine	12.0±0.0	14.0±0.0	12.0±0.0	13.0±0.0	18.0±0.0

Table 2. Average inhibition halos (mm) of crude extracts from plants by the technique of agar disk diffusion against *Candida* species after 48 hours incubation at 37 °C.

Plant	*C. krusei*	*C. albicans*	*C. glabrata*	*C. tropicalis*
Angico	8.0±0.0	0.0	0.0	0.0
Aroeira	0.0	0.0	0.0	0.0
Bom-nome	0.0	0.0	0.0	0.0
Embiratanha	9.0±0.0	9.7±0.6	8.0±0.0	0.0
Mororó	7.0±0.0	9.0±0.0	0.0	0.0
Quixabeira	0.0	0.0	0.0	0.0
Umbuzeiro	0.0	0.0	0.0	0.0
Urtiga-Branca	0.0	0.0	0.0	0.0
Nystatin	13.5±0.5	21.4±0.4	24.0±0.0	20.4±0.2

It was also observed that the solvent 70% ethyl alcohol (negative control) did not affect the antimicrobial activity, once when tested in pure, it did not promote formation of inhibition halo of bacterial growth for any of the strains. On the other hand, nystatin and chlorhexidine (positive controls) produced inhibition halos against all species analyzed.

Discussion

The use of medicinal plants in dentistry has been a promising alternative in prevention and treatment of different diseases of the oral cavity [6,12,13]. The mastic, for example, has been subject of much research in health, including studies of antimicrobial activity against different microorganism species of the oral cavity [12,13]. The present study confirmed the antimicrobial activity of the mastic extract, which proved being effective against the species *S. aureus* and *E. faecalis*. On the other hand, it showed no efficacy against *S. mutans* and *Candida* species, contrary to the findings of other studies [12,13]. The extract of urtiga-branca was the only one that showed no antimicrobial activity. It is possible that variations in the method of obtaining the extracts and the technique used to evaluate the antimicrobial activity produce positive results, different from those found in this study.

The mororó presented antimicrobial activity against *E.faecalis, S. aureus, C. Krusei* and *C. albicans*, the umbuzeiro and bom-nome against *E. faecalis and S. aureus*. These results support the use of these plants in medicinal use, which macerate or decoction of the stem bark of mororó has been used as a tonic, depurative and to treat diabetes [10], the umbuzeio in ophthalmic disorders and decoction of bom-nome in renal and ovarian inflammations and infections and external ulcers [8].

Few studies have been published revealing the bioactive properties of Quixabeira [14,15]. In this study, only Quixabeira showed antimicrobial activity against *S. aureus*. In a previous study using the well technique was checked activity of this plant against *E. faecalis* [16]. Divergence of results was also found with angico, whose results were positive in this study, differing from other results [8,17] found by the well method in agar medium, effectiveness of angico against all strains of *S. aureus* analyzed, verifying halos of up to 25 mm. These differences in outcome can be attributed to methodological variation, as well as the origin and characteristics of plant material [12].

Given the findings, we suggest to carry out more detailed research using different techniques to better evaluate the antimicrobial activity of medicinal plants.

Conclusions

Of the extracts tested, angico, umbuzeiro, the aroeira-da-praia and bom-nome showed antimicrobial activity against *E. faecalis*. None of the extracts showed action against *S. mutans* and *E. coli*. Against *Candida* species, only mororó, embiratanha and angico showed antimicrobial activity.

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